METHOD OF PRESERVING FOODSTUFFS AND BEVERAGES

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Detailed description of the invention

The present invention relates to a method of preserving foodstuffs and beverages, and of improving the quality thereof.

More specifically it relates to a method of preserving foodstuffs and beverages, and of improving the quality thereof by virtue of the addition of oxidation-reduction enzymes and bacteriolytic enzymes.

For the purpose of the present invention the term 'oxidation-reduction enzymes' is to be taken to signify oxidation-reduction enzymes having oxygen as a receptor. Examples include glycolate oxidase, lactate oxidase, glucose oxidase, hexose oxidase, galactose oxidase,

aldehyde oxidase, xanthin oxidase, pyruvate oxidase, oxalate oxidase, D-asparate oxidase, L-amino acid oxidase, D-amino acid oxidase and amine oxidase. Meanwhile, specific examples of bacteriolytic enzymes include lysozyme (EC 3.1.17), lysozyme salts, and enzymes produced by *Bacillus subtilis, Streptomyces griseovilens* and *Brevibacterium lytticum.*

All the oxidation-reduction enzymes used in the present invention react with a substrate to produce hydrogen peroxide, and are extracted and refined from animal organs, plants, micro-organisms and other sources.

For instance, hexose oxidase is prepared from unripe oranges, and glucose oxidase from a fungus of the genus Penicillium. On the other hand, L-amino acid oxidase and D-amino acid oxidase are obtained from microorganisms.

Of the bacteriolytic enzymes used in the present invention, lysozyme is an enzyme widely distributed in the animal and plant kingdoms which serves to hydrolyse mucopolysaccharide and mucopolypeptide beta (1–4) bonds. It is also produced by certain microorganisms, and a convenient variety for practical use is derived from egg-white. A single-chain polypeptide with a molecular weight of approximately 14,500 and an isoelectric point of 10.5–11.0, egg-white lysozyme has a distinctive sweet taste.

Meat and fish products, custards and ice-creams are examples of foodstuffs which present problems of preservation. Even where the use of preservatives is permitted, existing additives alone are not sufficient to provide a solution. Much more problematic is the case of milk, raw noodles, cream buns and similar foodstuffs where the use of preservatives is not permitted, and this is a perpetual source of concern for manufacturer and retailer alike. What is needed is a method of preservation which is safe and free from toxicity.

In view of the abovementioned circumstances the authors of the present invention have conducted painstaking research, as a result of which they have discovered that the desired aim may be attained through the combined use of oxidation-reduction enzymes and bacteriolytic enzymes.

That is to say they have discovered that the combined use of oxidation-reduction enzymes and bacteriolytic enzymes is much more effective than the simple use of oxidation-reduction enzymes, and it is this discovery which led to the perfection of the present

invention.

Thus, it is an object of the present invention to provide a novel method of preserving foodstuffs and beverages, and of improving the quality thereof.

The present invention may be applied to any foodstuffs or beverages, specific examples being meat and fish products, milk, butter, cheese, soft drinks, fruit juices, raw noodles, bean paste, custard, fresh cream, butter cream and ice-cream.

The present invention is implemented by adding the enzyme in the normal manner. There is no particular limit on the amount of enzymes added, but for each enzyme it is ideally within the range 5–500 ppm.

The action mechanism whereby the present invention effectively preserves foodstuffs and beverages is not entirely clear, but it is assumed that the oxidation-reduction enzymes allow the substrates to react with oxygen in such a manner as to generate hydrogen peroxide, and this kills any microorganisms which may be present in the foodstuffs or beverages. Further growth of aerobic microorganisms will then be inhibited as a result of reduced oxygen levels within the foodstuffs.

It should be pointed out that the antimicrobial strength of the hydrogen peroxide generated in the present invention is far in excess of that used in the bleaching and pasteurisation of foodstuffs.

There follow a number of embodiments with the aid of which the present invention will be described in yet greater detail.

Embodiments

Tryptosoy broth (pH 7.0) with differing oxidation-reduction enzyme contents and lysozyme concentrations ranging from 0 to 40 ppm was inoculated with various bacteria and cultivated at 30 °C for four days. Bacterial growth was observed macroscopically, and the results shown in Tables 1–3.

As will be seen from Tables 1–3, the oxidation-reduction enzymes and egg-white

lysozyme exhibited a synergic effect on three typical putrefactive bacteria.

Table 1 *E. coli*

		L-amino acid oxidase ppm			
		0	5	25	125
Egg-white lysozyme ppm	0	++	++	++	+
	10	++	+	–	–
	20	++	+	–	–
	40	++	+	–	–

Table 2 *B. subtilis*

		Glucose oxidase ppm			
		0	10	20	40
Egg-white lysozyme ppm	0	++	++	+	+
	10	+	–	–	–
	20	+	–	–	–
	40	–	–	–	–

Table 3 *Staph. aureus* 209P

		L-amino acid oxidase ppm			
		0	5	25	125
Egg-white lysozyme ppm	0	++	++	+	–
	10	++	+	–	–
	20	+	+	–	–
	40	+	+	–	–

<Embodiment 1>

To commercially available milk were added 20 ppm bacteriolytic enzymes produced by *Bacillus subtilis*, and 20 ppm glucose oxidase. The preservative effect was compared with that obtained when the two were added independently. The numerical values in the table below represent the total number of bacteria per litre of milk.

Table 4

	Duration of preservation (days)			
	0	2	4	6
No additive	3×10^2	7×10^4	7×10^6	6×10^8
Bacteriolytic enzyme (20 ppm)	3×10^2	8×10^3	1×10^6	2×10^8
Glucose oxidase (20 ppm)	3×10^2	4×10^3	3×10^5	3×10^7

Bacteriolytic enzyme + glucose oxidase	3×10^2	7×10^2	7×10^3	9×10^4

<Embodiment 2> *Kamaboko* [a sort of fish sausage]

A total of 10 kg mixed fish meat was rinsed, dehydrated and minced. To this was added 500 mg glycolate oxidase and 500 mg egg-white lysozyme. It was then mashed and mixed well before adding 300 g salt, 500 g sugar, 500 g defatted soybean meal, 30 g sodium glutamate and 200 mL *mirin* [sweet rice wine]. After further mashing, 1.5 kg starch and 3 L water were added, and it was mashed well again.

The resultant mashed fish meat was heaped on to small oblong wooden blocks, moulded and steamed for 15 minutes at 100 °C. It was then cooled to yield the finished product.

The *kamaboko* produced in this manner was left in a container at a constant temperature of 30 °C to see if the surface became sticky. This occurred after ten days as opposed to three days in the case of a control batch which had been prepared without the addition of glycolate oxidase, or eight days in the case of a batch prepared without addition of egg-white lysozyme.

<Embodiment 3> *Udon* [a sort of noodle]

To 3.7 kg wheat flour were added 1.4 L 15% salt water, 450 mg galactose oxidase and 450 mg egg-white lysozyme. This was mixed well and kneaded. It was then rolled out and cut before being boiled in water and formed into portions of a specified weight.

The portions produced in this manner were still eatable after being stored at 30 °C for 60 hours, whereas a control batch produced without the addition of preservatives went off and became uneatable within 24 hours. Meanwhile, a batch produced in the same manner but without the addition of egg-white lysozyme went off and became uneatable within 48 hours. It was therefore clear that the combined use of an oxidation-reduction enzymes and egg-white lysozyme was highly effective.

<Embodiment 4> Custard cream

Custard cream was made from the following ingredients.

Glucose	100 g

Sugar	700 g
Wheat flour	100 g
Cornflour	100 g
Eggs	30
Vanilla flavouring	Small amount
Milk	2 L
Hexose oxidase	100 mg
Egg-white lysozyme	60 mg

This was compared functionally and for the number of bacteria with a control to which hexose oxidase and egg-white lysozyme had not been added.

The results are shown in Table 5.

Table 5

	Number of days elapsed				
	2	3	4	5	6
Control	–	+ 10^7	++ 2×10^8		
Hexose oxidase + lysozyme	–	– 6×10^4	– 6×10^5	– 7×10^6	± 8×10^7

(57) Claim

A method of preserving foodstuffs and beverages, and of improving the quality thereof characterised in that oxidation-reduction enzymes and bacteriolytic enzymes are added to the foodstuff or beverage.

(56) Reference

US Patent 3193393